Coastal Bioluminescence: A Collaboration on Sources, Population Dynamics and Critical Instrumentation

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LONG -TERM GOALS

Our long-term goals center on understanding the mechanisms and adaptive significance of all forms of marine bioluminescence (BL). Within that broad scope, emphasis is on physically excitable coastal BL because of its relevance to Navy interests and because the complex structure of coastal zone biota invites study of the impact of BL on population dynamics. Recognizing the need for BL detectors for use on the many platforms employed in coastal zone research and Navy survey work, we have made a major effort to develop and demonstrate reliable, versatile and convenient instrumentation for this work. With continuing deployments of the Multipurpose Bioluminescence Bathyphotometer (MBBP-G3) from several platforms in various environments, we expect our understanding of the geography and seasonal variability of BL in the coastal environment to improve significantly.

OBJECTIVES

During this award period we continued to work towards the following objectives:

1. Characterize variability in the BL signal and explore the relationship between signal properties and planktonic species structure. This may ultimately lead to taxonomic discrimination of at least some components of the bioluminescent population and thereby further increase the reliability of estimates of longer term predictions.

2. Improve extent of long-term deployments of the MBBP-G3, particularly with regard to counteracting the effects of possible biofouling of the detection chamber on the BL signal.

3. Evaluate the performance of the MBBP-G3, especially with respect to its long-term stability in moored systems and in comparison with other instrumentation, particularly the Navy standard HIDEX.

4. Transition the MBBP-G3 BL sensing technologies from the research to the commercial realm to achieve more widespread temporal and geographic coverage with reliably uniform instrumentation from a wide variety of platforms.

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WORK COMPLETED/RESULTS

1. Continued integration of MBBP-G3 onto a variety of platforms. These include:

    a. A fleet attack submarine, arranged by Mr. Mark Geiger, NAVO.

    b. Integration on the ORCAS profiler for the 2006 Layered Organization of the Coastal Ocean (LOCO) experiment, Monterey Bay, CA, in collaboration with Drs Van Holliday, Percy Donaghay and Jim Sullivan.

    c. Part of the standard payload on the Dorado AUV (MBARI) to conduct periodic surveys in Monterey Bay, CA, in collaboration with Dr. Steven Haddock. This AUV also collected BL data during the 2006 LOCO experiment.

    d. On an APEX coastal profiling test mooring in collaboration with Dr. Edith Widder, who is establishing an environmental monitoring network along the east coast of Florida.

    e. On an autonomous profiler mooring, Avila Beach, CA, in collaboration with Mark Moline (California Polytechnic State University).

2. Our long-term moored deployments off the coast of Santa Barbara as part of the Network for Environmental Observation of the Coastal Ocean (NEOCO mooring, Stearns Wharf, Santa Barbara, CA) and Multi-disciplinary Ocean Sensors for Environmental Analysis and Networks (MOSEAN) CHARM mooring located in shallow depths off La Conchita, CA. These deployments provide information on seasonal and geographic variability in the BL signal as well as providing a test bed for developing anti-biofouling technology.

3. To improve understanding of the variability in the BL signal in relation to the variability in the species content of the plankton population, experiments were continued at the Cal Poly pier facility in collaboration with Mark Moline (California Polytechnic State University).

4. Preliminary analysis of the BL signal has been conducted with a MBBP-G3 containing a transparent 3-turn acrylic helix to improve and normalize the specimen residence time within the detection chamber.

5. Initial steps on refurbishment of the (High-Intake Defined EXcitation) HIDEX-Generation 1 for anticipated comparison studies of the MBBP-G3 against the Navy standard.

RESULTS

As the MBBP-G3 is proven conveniently adaptable to a variety of platforms, bioluminescence as an oceanographic measurement may become more commonplace, especially with the availability of an appropriate commercial unit. Continued platform integrations of the MBBP-G3 provide an opportunity to expand the adaptability of this technology and extend the database of BL data collected at a variety of geographic locations during various seasonal and oceanographic conditions. To expand the breadth of platforms in which the MBBP-G3 has been installed, one of our systems was incorporated into the ORCAS profiler, deployed at 36.55.947 N, 121.55.329 W. The importance of the inclusion of BL measurements emerged during the July 2006 Layered Organization in the Coastal Ocean (LOCO), field experiment in Monterey Bay, CA. During July 14 to 28 the profiler defined a thin layer, in conjunction with other measurements of biological variability, namely chlorophyll concentration (Figure 1). One intensely bioluminescent thin layer was observed on the night of July 18th (decimal day 199), which is not correlated with a large chlorophyll signal. A possible explanation could be presence of a heterotrophic bioluminescent dinoflagellate population such as Protoperidinium, which has been observed in Monterey Bay, CA, during this season. Inclusion of highly luminescent species in a thin layer may well have determinative effect on recruitment and/or exclusion of other species in the layer.

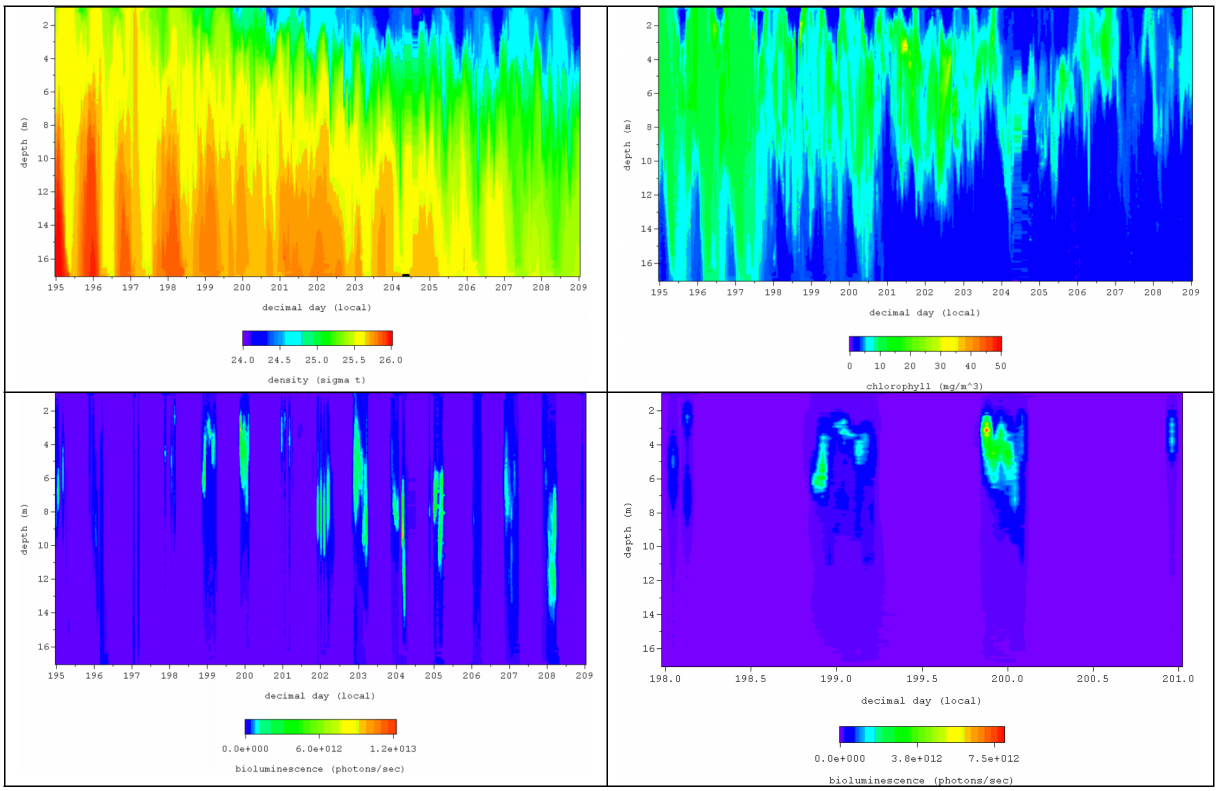

Figure 1: Data collected from the ORCAS profiler during July 14 (Julian Day 195) to July 28 (Julian Day 209). A. Density, B. Chlorophyll, C. Bioluminescence, D. Close up of the intense bioluminescent thin layer observed on July 18 and 19, 2006.

The MBBP-G3 has been part of the standard science payload on the Dorado AUV since 2002. During December, 2005 BL was measured in conjunction with density, temperature, salinity, nitrate, oxygen concentration, optical backscatter at 470 & 676 nm, and chlorophyll fluorescence as part of the monthly transects conducted in Monterey Bay, CA (Figure 2). These transects provide vital information on spatial and temporal changes in the bioluminescence signal within Monterey Bay and are collected in collaboration with Dr. Steve Haddock of MBARI. During the December 16 transect BL was observed to be strongest near the coast correlated with chlorophyll fluorescence. This is indicative of a population of a bloom of bioluminescent autotrophs, especially with the lack of surface nitrate presumed to have been used as the bloom began and matured. In contrast, BL observed offshore was not associated with chlorophyll fluorescence. This indicates a difference in the composition of the offshore bioluminescent population, in comparison to the nearshore one. Common to this region are heterotrophic bioluminescent populations, such as Protoperidinium, which are bioluminescent but do not fluoresce.

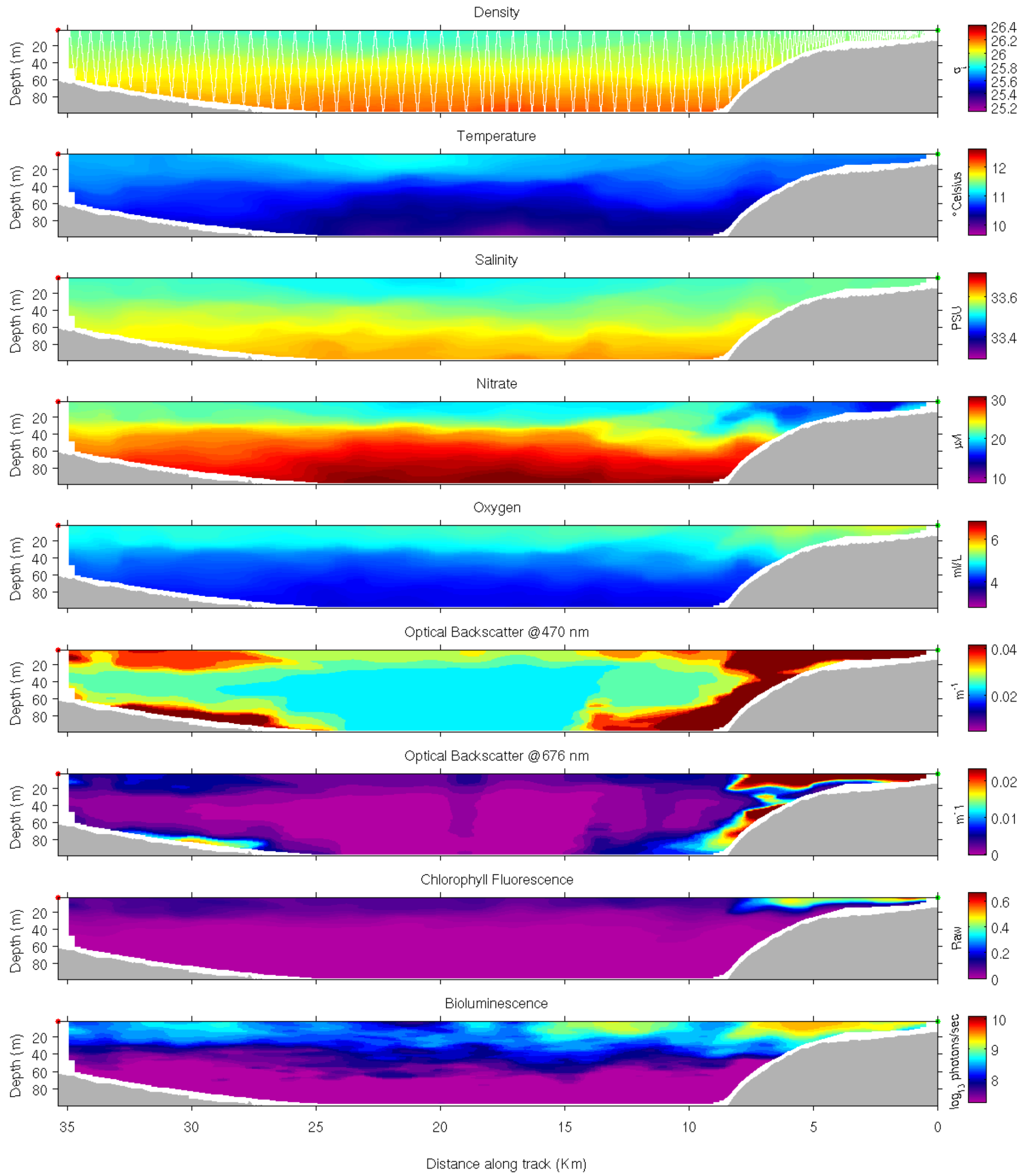

Figure 2: Monthly transect of Monterey Bay, CA on December 16, 2005. Transects began (red dot) in south Monterey Bay, near Pacific Grove, CA, continued to the M1 mooring in the center of the bay and ended in north Monterey Bay, near Aptos, CA (green dot). Measurements were made of the following: Density (σ^T), temperature (° Celsius), salinity (PSU), nitrate (μM), oxygen concentration (mL/L), optical backscatter (m^{-1}) at 470 & 676 nm, chlorophyll fluorescence (raw counts), bioluminescence (log_{10} photons/sec). Two distinct regions of BL can be seen: nearshore and offshore.

Dr. Edith Widder, of Ocean Research and Conservation Association, Ft. Pierce, FL., and engineering staff at Harbor Branch Oceanographic Institute (HBOI) are currently in the process of installing a MBBP-G3 on an APEX profiler mooring. This opportunity provides yet another novel platform for the MBBP-G3 to record BL in a variety of geographic locations. Dr. Widder will use this semi-autonomous drifting profiler to search for high levels of BL in the water off the E. Florida coast. The profiler uses an Ultra-Short Base Line (USBL) tracking system to maintain its position. At a specific location in the water column the profiler will take multiple samples, to measure BL, salinity and temperature which are reported to shore at predetermined intervals. Brian Ramos, engineer at HBOI, has reported the completion of the housing which will contain the MBBP-G3 (Figure 3) and is attached to the bottom of the drifter with data and command interfacing.

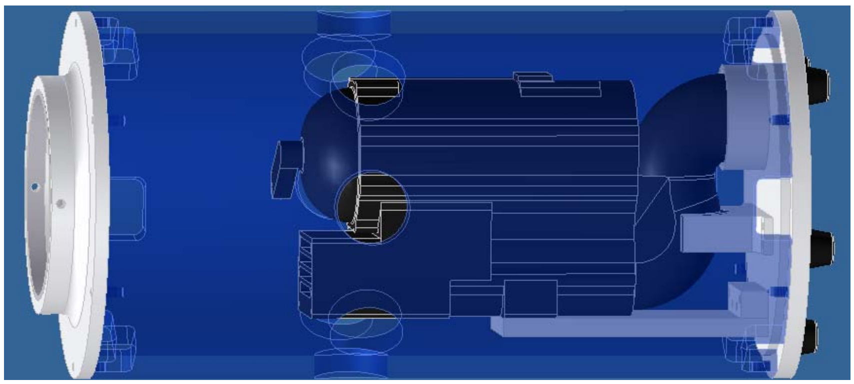

Figure 3: Housing that will attach to the bottom of the APEX profiler to house the MBBP-G3 shown in black with inlet in upper right.

Over short term deployments, such as with the ORCAS profiler or Dorado AUV, biofouling is not a great concern. However during longer deployment periods of 1-3 months, biofouling plays a significant roll in reducing the BL signal and requires mitigation. In the detection chamber of the MBBP-G3 growth of organisms on the reflective inner surface of the detection chamber will reduce its reflectivity. Flow rate, which provides the turbulence necessary to induce BL excitation in organisms as they enter the detection chamber will also be reduced. As biofouling continues, there will be a significant decrease in the BL signal. To try to reduce biofouling, several devices were fabricated and tested on long-term deployment platforms, in locations where the rate of biofouling differed: 1. Stearns Wharf, Santa Barbara (NEOCO) where rapid and massive fouling will occur in an unprotected instrument, and 2. CHARM mooring, off La Conchita, CA in < 25 m of water where fouling is present but more gradual in onset, as indicated by a gradual diminishment of flow recorded during three month deployment periods. Since the CHARM mooring is a platform powered only by batteries, power usage is also an important consideration favoring development of antifouling technology.

Taking advantage of the design of the BP, a passive method was developed to flood the detection chamber with copper ions when data was not being taken. A device was fabricated to exploit the chamber pressure during pumping by diverting a fraction of the flow into an expandable bag filled with copper mesh. Upon cessation of pumping during a measurement cycle the overpressure in the bag expels the collected water partially saturated with copper ions back into the detector chamber from which it diffuses slowly enough out through the chamber opening to reduce fouling markedly before the next pumping cycle. This scheme is simple and requires little modification of the system. This antifoulant technology has produced successful results over the last 18 months and the design has been steadily improved. Results from previous deployments are evidenced in the following images (Figure 4).

Figure 4: A. MBBP-G3 after long-term deployment at the NEOCO site, without use of antifouling technology during deployment with evidence of heavy biofouling. B. MBBP-G3 detection chamber after long-term deployment, using passive diaphragm technology to deliver a toxic dose of copper ions into the detection chamber between measurement cycles.

Recent inspections of the MBBP-G3 after long-term deployment showed that sediment deposition was not uniformly distributed on the walls of the chamber. In order to understand why this was the case, more flow visualization tests were done, using a clear chamber. Previous tests of flow turbulence were conducted with an intensified camera looking down the length of the detection chamber and seeing an aggregative signal indicating a generally helical flow. However the uneven sediment distribution indicated that the flow path was not wholly helical. Several paths including the most desirable helical seemed likely. Therefore, a helix fabricated out of acrylic (PMMA) was installed in the chamber to force a uniform flow path. The helix used was designed to have 3 turns, from the point of introduction into the detection chamber from the inlet to the outlet (Figure 5). The composition of the helix is expected not to interfere with the magnitude of BL because the reflective index of the acrylic is similar to water. Flow visualization tests with the helix in place were much more satisfying. Currently, a helix equipped BP is currently deployed on the CHARM and NEOCO moorings and we anticipate this modification will be included in the commercialized version.

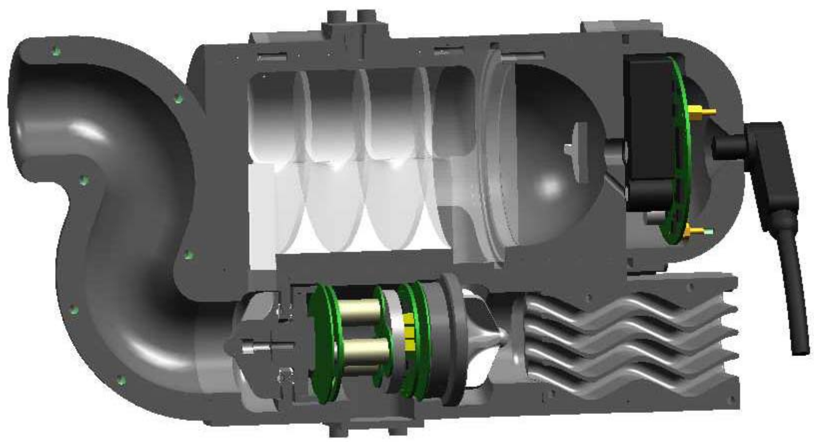

Figure 5: MBBP-G3 with clear 3-turn helix inserted into the detection chamber to normalize chamber flow.

IMPACTS AND APPLICATIONS

The MBBP series of BPs presently appears to be the only rigorously evaluated, pumped small BPs suitable for interchangeable coastal operations from moorings, profiling systems, towed systems and AUVs (Case, et al, 1993). The unique design of its flow path and high data rate allows for visual "on the fly" discrimination between major plankton sources and reliable intercomparison between instruments deployed from many different platforms.

To our knowledge, no other bathyphotometer has been incorporated into so many platforms, suggesting BL measured from instruments of this type might well become a commonplace measurement in optical and biological oceanography, rather than its present rarity. Its extensive use and ease of integration on a variety of platforms has attracted commercial interest that we hope will lead to an inexpensive BL-optics package making access to BL data more accessible to the entire oceanographic community.

Our involvement with both the NEOCO and CHARM moored systems, as well as with the CalPoly autonomous profiling mooring, are models taking us somewhat closer to our objective of having a widespread coastal network of bathyphotometers integrated with complimentary observational programs, thus to commence building up a long-term BL prediction program.

TRANSITIONS

In collaboration with our commercial partner WET Labs, we have completed the objectives of the STTR Phase I and Phase I Option I objectives: 1) To transfer G3 bathyphotometer design and technology from UCSB to WET Labs including the assimilation of mechanical and electrical design as well as software and control code. 2) To conduct a review of the bathyphotometer design in a forum to foster design improvements by a core group of bioluminescence researchers, providing an opportunity to determine the utility of integrating other IOPs with the BL sensor. 3) To develop a timeline for the WET Labs prototype construction (Underwater-Bioluminescence Assessment Tool, U-BAT) and 4) To define laboratory and field tests that will be used to characterize U-BAT, provide comparison to MBBP-G3 model and help to define final specifications for the construction of the commercial version of U-BAT. With the completion of these objectives, a preliminary embodiment design and calibration method have been defined for the commercial U-BAT and forms the basis for the STTR Phase II work, which has recently commenced.

RELATED PROJECTS

At no direct expense to ONR, work continues on firefly bioluminescence using laboratory resources when available. Using our locally developed integrating sphere, normal photic communication was measured in the laboratory between tethered flying males and perched females of *Photinus pyralis,* a species in which the flying male exchanges flashes with a perched female. This work represents the first accurate measurements of light emission in the spontaneous photic exchanges in any BL communication system, marine or terrestrial (Case, 2003; Case and Hanson, 2003). The investigation also revealed a hitherto unknown flight communication mode of the female that is important in understanding evolution of firefly photic communication, we suggest, from an ancestral alarm function.

Our laboratory continues a long-standing and productive study of BL brittle starfish with Prof. J. Mallefet, University of Louvain (Mallefet, et al 2003). His work in collaboration with J. Hendler, of the Los Angeles County Museum, has included a description of a new species, first discovered propagating in our laboratory aquaria!

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